Spatio-temporal analysis of yeast cells responses on a digital microfluidic platform

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Single cell analysis (SCA) has obtained great importance among biologists for elucidating cell-to-cell differences that are difficult to recognize in bulk measurements. Flow cytometry is one of the most widely used techniques to characterize non-adhering cells, however, its inability to analyze cells with spatio-temporal resolution is a major drawback. Continuous monitoring and analysis of non-adhering cells requires involvement of certain trapping systems for the precise manipulation and retention of these cells at a desired location. To date, various microfluidic platforms have demonstrated high-throughput single cell studies of non-adhering cells by implementing cell-specific trapping strategies[1,2]. In this study, we demonstrate the potential of electro-wetting-on-dielectric based digital microfluidics (EWOD-DMF), as automated and miniaturized systems to trap single yeast cells and analyze their responses towards antifungal treatment at a single cell resolution over time.

A DMF chip is illustrated for transporting discrete reagent droplets sandwiched between the two plates using electrowetting-on-dielectric (EWOD) actuation principle (Fig.1(a,b)). In addition, a microarray containing 21,000 cylindrical cavities (5 µm diameter and 3 µm deep) is fabricated in the top plate Teflon layer in a such way that the walls of the cavity remain hydrophobic whereas, the cavity bottom is hydrophilic. The on-chip cell trapping is achieved by allowing the cells to sediment on the array for 10 minutes. This is followed by subsequently transporting the cell droplet over the microarray multiple times (referred to as transport cycles). During this time, the cells enter the microwells by capillary forces and are trapped in the hydrophilic region of the cavities (Fig. 1(c)). The trapped cells are then subjected to antifungal treatment (Amphotericin B; AMB) and monitored over a period of 4 hours.

The effect of the number of transport cycles on cell trapping efficiency and cell viability was analyzed using propidium iodide. The decrease in trapping efficiency observed at higher transport cycles, indicates the removal of previously trapped cells and is possibly due to the strong shear forces that originate from transporting the cell droplet multiple times. In line with this hypothesis, a decrease in the viability of trapped cells was observed while increasing the number of transport cycles. Secondly, the response of trapped cells towards treatment with 100 µM AMB were investigated. Time-dependent killing was observed and approximately 100 % cell death was reached after 4 hours (Fig. 2(b)). To the best of our knowledge, this is the first such demonstration of the use of EWOD-based DMF chips for in vitro cytotoxicity assays on single yeast cells with spatio-temporal resolution. More importantly, this platform can be used for thorough screening of newly developed antifungals in a semi high-throughput manner.

[1]E. Brouzes, M. Medkova, N. Savenelli, D. Marran, M. Twardowski, J. B. Hutchison, J. M. Rothberg, D. R. Link, N. Perrimon, M. L. Samuels, *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 14195–200. ; [2] C. Liberale, G. Cojoc, F. Bragheri, P. Minzioni, G. Perozziello, R. La Rocca, L. Ferrara, V. Rajamanickam, E. Di Fabrizio, I. Cristiani, *Sci. Rep.* **2013**, *3*, 1258. ; [3] D. Witters, K. Knez, F. Ceyssens, R. Puers, J. Lammertyn, *Lab Chip* **2013**, *13*, 2047–54.

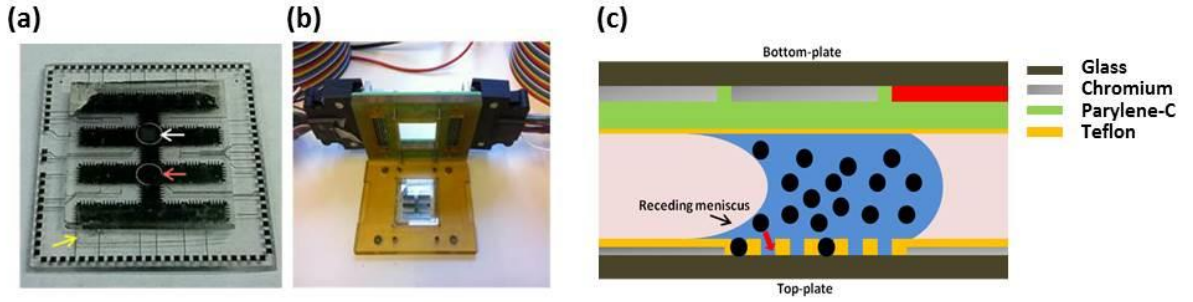

Fig. 1. EWOD-DMF assembled platform. (a) Depiction of yeast cell droplet under the microwell array (white arrow) and antifungal drug (AMB) droplet (red arrow) sandwiched between the top-bottom plate along with a spacing unit (yellow arrow); (b) Illustration of the DMF-interface unit ;(c) Schematic of the flipped DMF setup that is used to trap single cells.

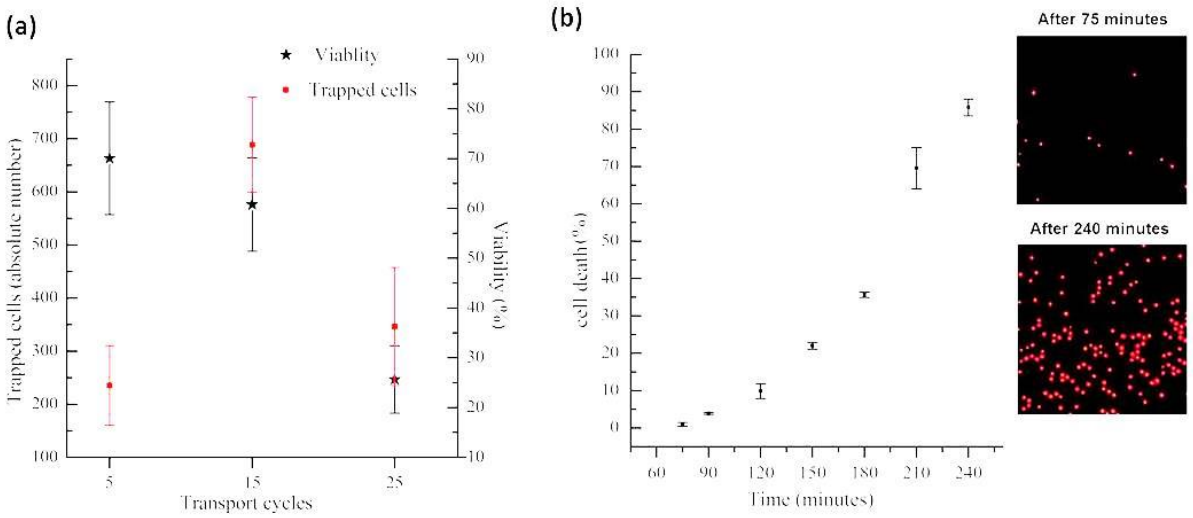

Fig 2. Antifungal treatment of *S. cerevisiae* cells on a EWOD-DMF platform. (a) Trapping efficiency and viability of cells over three transport cycles; (b) Occurrence of fungal cell death during treatment with 100 µM AMB over 240 minutes. Error bars indicate standard error of two replicates. (Inset) Microscopic images of dead cells (red spots) treated with 100 µM AMB after 75 minutes (top) and 240 minutes (bottom) of treatment.